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ORIGINAL ARTICLE

Can a hypercholesterolemic diet change the basal brain electrical activity and during *status epilepticus* in rats?

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Abstract

The brain is an organ rich in lipids, including cholesterol, in which these lipids are associated to structure and brain function. Thus alterations in lipid levels of diets may interfere in the brain electrical activity. Our aim was to evaluate the interference of hypercholesterolemic diets in the brain electrical activity in normal individuals and with epilepsy. Histological analysis and electrocorticograms (ECoG) were performed in animals fed with and without hypercholesterolemic diet before and during the *status epilepticus* induced by pilocarpine. The power spectrum of ECoG was used to estimate the contribution of different brain rhythms in ECoG signal. The animals submitted to the *status epilepticus* showed cell death, vacuolization with destructuration of the cell layers. Both animal groups, those with *status epilepticus* and *status epilepticus* with hypercholesterolemic diet, showed cellular lesions similar. The hyperlipid diet promoted increase of brain electrical activity, this was revealed by increase in the average power of beta wave (14–30 Hz) and decrease in the average power of the delta wave (0,5–4 Hz). This increase of brain electrical activity was even higher when the animals were fed a hypercholesterolemic diet and submitted to *status epilepticus*. Animals fed with hypercholesterolemic diet and submitted to *status epilepticus* presented a higher increase in brain excitability compared to control animals. We observed that hypercholesterolemic diet favored a greater severity of the *status epilepticus*.

Keywords Cholesterol · Power spectrum · Status epilepticus · Brain electrical activity

Introduction

Cholesterol is an essential component of cell membranes and it is important in maintaining the physico-chemical properties of these membranes, such as membrane fluidity and permeability (Zhang and Liu 2015). The myelin sheath that surrounds neurons is formed by type of glial cell, which confer high proportions of lipids, including cholesterol, in the nervous system.

High lipid indexes in the brain composition indicate the importance of these molecules for nervous system structure and function. The brain is composed by 80% of lipids that correspond to about 25% of whole body cholesterol (Björkhem and Meaney 2004). The lipids are mainly concentrated in the myelin sheath where they play the role of electrical insulation (Dietschy and Turley 2004), so presenting importance in the adequate propagation of postsynaptic and action potentials.

Cholesterol is particularly concentrated in microregions called lipid rafts, which serve as organizer to membrane receptors and ion channels in neurons. Studies have reinforced this interaction between rafts and membrane receptors showing their importance in regulating the function of these receptors (Burger et al. 2000; Pucadyil and Chattopadhyay 2006). Changes in the levels of theses lipids can alter the functional behavior of ion channels and consequently the brain

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excitability. Studies also have reported that cholesterol is essential to neuronal plasticity (Mauch et al. 2001; Goritz et al. 2002; Klopfenstein et al. 2002; Barres and Smith 2001).

Organic cholesterol levels are maintained by both cell production and what is provided in the food. Western diet, high in cholesterol and saturated fats, has been increasingly consumed (Vitali et al. 2014). Obesity and high plasma levels of cholesterol and triglycerides are consequences of this diet, moreover studies have linked its consumption to Alzheimer's disease and to other forms of cognitive disorder (Di Paolo and Kim 2011). The brain is an organ rich in lipids, in which these lipids are directly associated with structure and brain electrical function. Thus alterations in lipid levels of diets may interfere in the cerebral dynamic, causing alterations in the tissue excitability and consequently in the brain electrical activity.

Electrocorticogram (ECoG) is used to analyze the brain electrical activity, recording voltage variation by time. Postsynaptic and action potentials in a neurons population are responsible for the electrical activity detected by ECoG. Although these records seem random, it is possible to identify cerebral rhythms that vary according to the brain area, emotional state and consciousness. The most important brain rhythms are: delta (0,5–4 Hz); theta (4–8 Hz); alpha (8–14 Hz); and beta (14–30 Hz) (Guyton and Hall 2006; Pessoa et al. 2016). Greater contribution of higher frequency waves increases the speed of brain activity, promoting greater excitability. Changes in electrical activity may indicate disorder, such as epilepsy (Guyton and Hall 2006).

Epilepsy is a neurological disease characterized as a synchronous and recurrent hyperexcitability (Cukiert 2006). Thus, epileptic patients are more sensitive to changes in brain excitability. Despite all the knowledge about the performance of brain cholesterol, studies still diverge on the action of diets high in lipids on cerebral excitability in healthy individuals and with epilepsy. Some diets high in lipids, such as ketogenic diets, are indicated as an alternative treatment for epilepsy in order to decrease the own excessive excitability of disease (Nordli and De Vivo 1997; Nordli 2002; Kossoff 2004). However, other studies indicate that excess brain cholesterol can lead to the death of hippocampal interneurons, thus increasing cerebral excitability (Chali et al. 2015).

In view of these divergences, our aim was to evaluate the interference of hypercholesterolemic diets in brain electrical activity in both normal individuals and with epilepsy.

Materials and methods

Animals and experimental design

40 male Wistar rats were utilized to assay. The rats were housed at controlled temperature (23 ± 2 °C), humidity (50%) and a 12-h light–dark cycle with food and water *ad libitum*

Assay was carried out in accordance with procedures approved by the Local Committee for the Care and Ethical Use of Animals in Research (CEUA/UFRPE, Recife, PE, Brazil), outlined in protocol number 103/2014 – CEUA/UFRPE.

Animals were divided into two groups. Group 1 ($n = 20$) animals were fed a standard balanced diet (Presence, Betel/Paulínia, São Paulo, Brazil). Group 2 ($n = 20$) animals were fed a hypercholesterolemic diet (25% of pig fat and 75% of standard diet) from 60 to 120 days postnatal. At 120 days of age, 10 animals from each group were used for histological analysis, and the others were used to record the brain electrical activity.

Plasma cholesterol tests

This test was done with purpose to monitory the plasma cholesterol levels of animals submitted to hypercholesterolemic diet. The test allows evaluating the diet effectiveness. The animals´ blood samples (0.5 ml) were collected to measure the total plasma cholesterol level by the equipment Accutrend Plus (Roche, Amadora, Lisboa, Portugal).

Histological and electrophysiological analysis

The animals were divided into 4 groups for the histological analysis: group 1 - control animals, group 2 - animals submitted to *status epilepticus* for a period of 4 h, group 3 - animals fed a hypercholesterolemic diet and group 4 - animals fed a hypercholesterolemic diet and submitted to *status epilepticus* for a period of 4 h.

The brains of the animals were collected to histological analysis the coronal hippocampal sections according to Pessoa et al. (2017). The sections were stained with cresyl violet (Nissl staining). Photomicrographs of hippocampus regions were obtained at 40X to 400X magnification: CA1, CA2, CA3, the dentate gyrus and the hilus of the hippocampal formation.

The procedure of electrodes implantation and recording of the brain electrical activity of animals were realized according to Pessoa et al. (2017). In short, the

animals with 115 days of age were anesthetized by an association of ketamine (50 mg) and xylazine (20 mg) at a dose of 0.1 ml / 100 g body weight. A longitudinal skin incision was made on the midline of the skull, after two orifices were made by a small drilling machine and two surgical screws were placed on the left hemisphere. A screw was placed in the parietal area of the sensorimotor cortex and another screw was placed on the frontal bone (reference electrode). Five days after electrodes implantation, the animals were placed in a Faraday cage to record the brain electrical activity. The ECoG recording was performed by a 410C EMG device (EMG System do Brasil, São José do Campos, São Paulo, Brazil). The ECoG was recorded for 30 min (baseline). After the first 30 min of recording, 350 mg/kg of pilocarpine hydrochloride (Sigma-Aldrich, St. Louis, Missouri, USA) were administered to induce *status epilepticus*, and the recording was continued for another 30 min, resulting in 60 min of recording. The animals were euthanized by anesthesia after the ECoG recording.

The signal filtering and the ECoG analysis were analyzed using MATLAB 7.8 software (MathWorks, Natick, Massachusetts, USA). The ECoG was recorded for 30 min, segmented into 2 min windows, for each experimental condition. Altogether, 15 segments were obtained for each experimental condition, totaling 30 segments per animal. The segments were imported to the MATLAB 7.8 software, and an algorithm for obtaining the power spectrum of the brainwave was implemented (Pessoa et al. 2016).

Mathematical analysis: Fourier transform and power Spectrum

The Fourier Transform (FT) is a method that determines the contribution of each frequency component in a time series. The function F(f) is the FT of the temporal function f(t), which represents amplitudes of different wave frequencies that compose the signal f(t); transforming time-domain data to frequency domain data. F(f) represents the frequency components contribution of f(t), as demonstrated by following equation (Welch 1967; Weisstein 2004).

$$F(f) = \int_{-\infty}^{\infty} f(t) e^{-i2\pi ft} dt \quad (1)$$

FT square of the ECoG originates its power spectrum. Average power value obtained by power spectrum allows estimating the contribution of different brain rhythms in ECoG signal. Power spectrum for an ECoG signal can be calculated as follow:

$$\overline{E}_{\omega} = \frac{\int_{f_s}^{f_e} |F(f)|^2 df}{\int_{f_s}^{f_e} df} \quad (2)$$

Where F(f) is the FT of the f (t) signal, here represented by the ECoG. The $\overline{E}_{\omega}$ is the power spectrum normalized by a determined frequency interval $\omega = [fs, fe]$, here represented by the different rhythms (Welch 1967).

Statistical analysis

The Mann–Whitney U test was used to compare the independent data of cholesterol. The Wilcoxon test was used to compare the paired data of the average power values of ECoG signals. Results are expressed as median and interquartile range. P values ≤0.05 were considered statistically significant.

Results

3.1 Plasma cholesterol levels

The hypercholesterolemic diet was monitored by total plasma cholesterol test in the animals. The animals fed with hypercholesterolemic diet showed plasma cholesterol levels (165.0 ± 11.0) significantly higher than the control animals (153.0 ± 3.0, $p = 0.004$). The diet promoted an increase of 7.8% in relation to the plasma cholesterol of control animals.

Histopathological analysis

In the histopathological analysis of hippocampal region of the animals submitted to the *status epilepticus* was noted cell death, vacuolization with destructuration of the cell layers, mainly in the areas CA1, CA2 and CA3 (Fig. 1). Moreover, were observed neuronal necrosis and gitter cells (cells that realizes phagocytosis of dead neurons) (Fig. 2). Both animal groups, those with *status epilepticus* and *status epilepticus* with hypercholesterolemic diet, showed cellular lesions similar (Fig. 1).

Brain electrical activity

The animals fed with hypercholesterolemic diet showed higher average power value of beta wave ($p = 0,01$), faster wave, and lower average power value of delta wave, slower wave, compared to control animals (Fig. 3 and Table 1). These values indicated that the supplementation with hypercholesterolemic

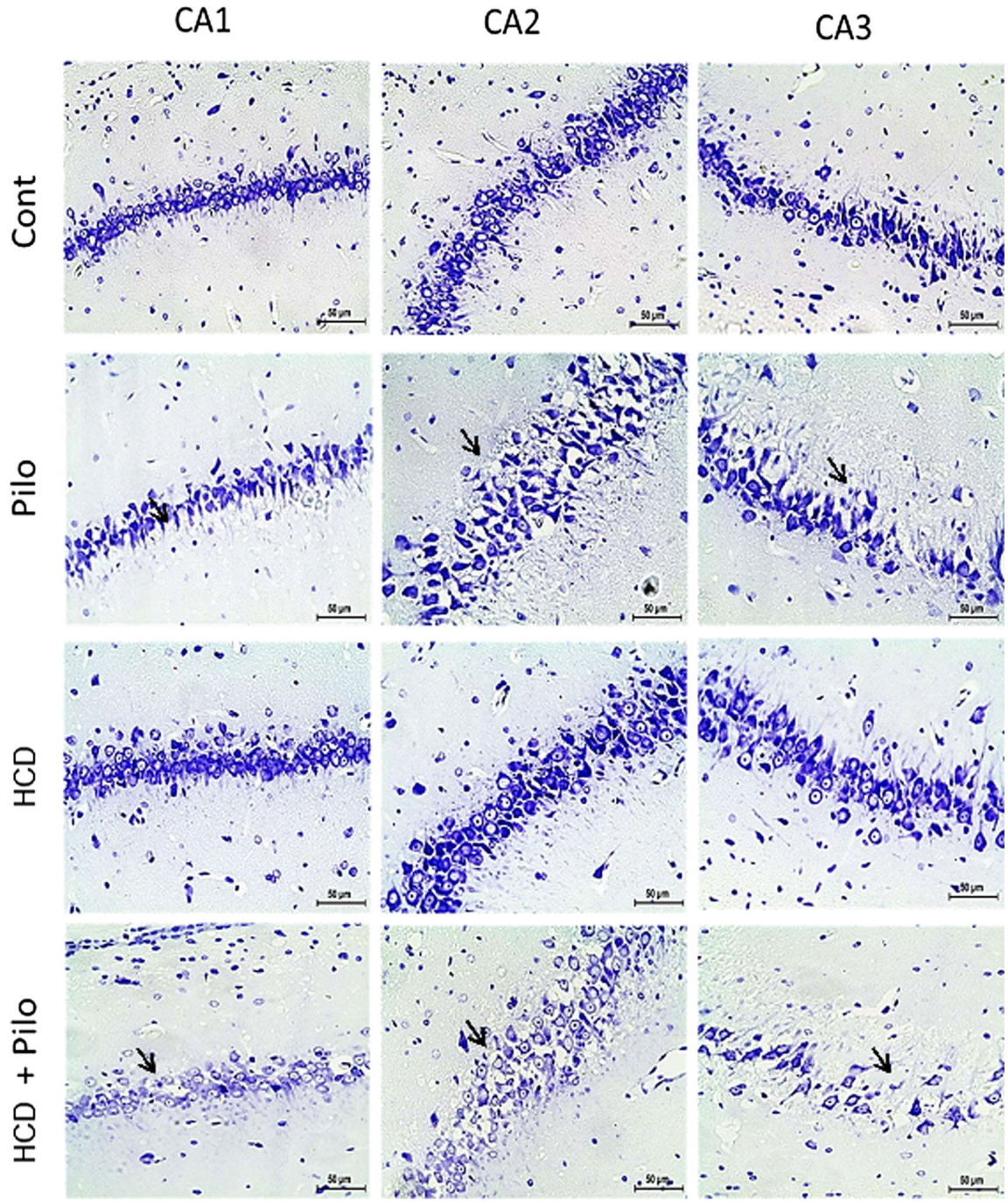

Fig. 1 Area CA1, CA2 and CA3 of rat hippocampus in coronal section: control animals (Cont); animals submitted to *status epilepticus* through pilocarpine (Pilo); animals fed with hypercholesterolemic diet (DHC); animals fed with hypercholesterolemic diet and submitted to *status epilepticus* (DHC + Pilo). Arrows indicate vacuolization provoked by neuronal death (Nissl staining)

diet promoted an increase in brain excitability. The average power values of theta and alpha waves did not have significant differences.

The animals fed with hypercholesterolemic diet and submitted to *status epilepticus* show higher average power values of beta wave ($p = 0.01$) in relation to its baseline (Table 1), presenting an even greater increase in brain excitability. These animals also presented higher values of alpha wave ($p = 0.01$) and beta ($p = 0.04$) compared to animals without hypercholesterolemic diet (Table 1, Fig. 4). This result indicates that pilocarpine elicited a more pronounced excitatory response in animals fed with hypercholesterolemic diet.

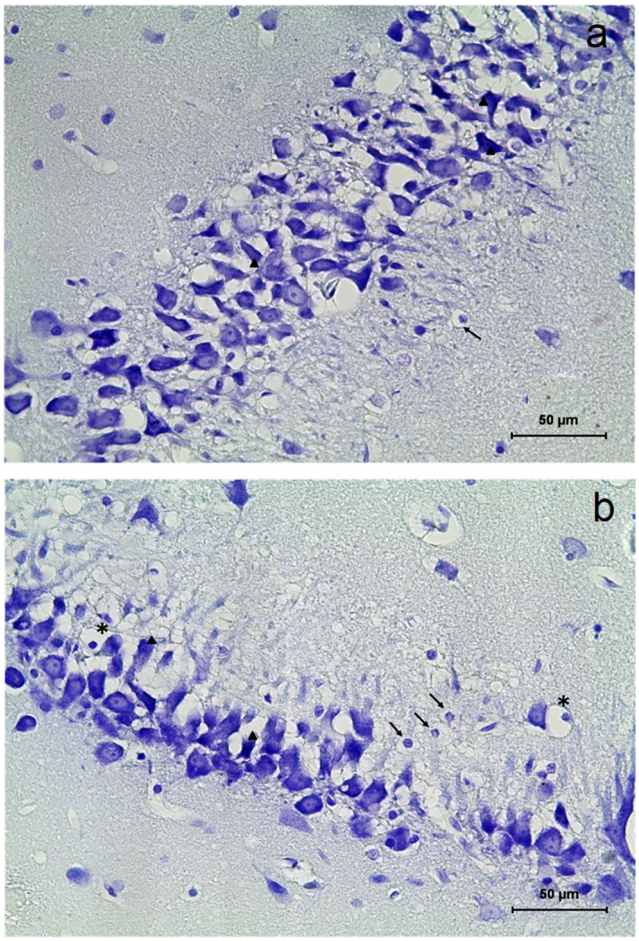

Fig. 2 Hippocampus coronal section of rat treated with pilocarpine showing lesions provoked by *status epilepticus* in the areas CA2 (**a**) and CA3 (**b**). The images show: arrows indicate gitter cells, triangles indicate neuronal death and asterisks indicate neuron phagocytosis (Nissl staining)

Discussion

We have observed that *status epilepticus* induced by the pilocarpine caused neuronal death in the hippocampal region, especially in the area CA1, CA2 and CA3. The hippocampal lesion promoted by pilocarpine is well described in Ferrari et al (2008). Nevertheless, we showed that the animals fed with hypercholesterolemic diet presented hippocampal damages more severe when submitted to *status epilepticus*. However, our results did not indicate lesions derived exclusively from hypercholesterolemic diet consumption, as reported by Chali et al. (2015).

The increase in the mean power of the beta wave, faster wave, in the basal ECoG of the animals that received hypercholesterolemic diet indicated a greater brain electrical activity. Because of the various levels that cholesterol can act in the nervous system, it is difficult to infer the mechanism by which cholesterol has elevated the brain activity. However, some hypotheses may be proposed.

One of them, the cholesterol that presents a nucleus with a flat structure and rigid that hydrophobically interacts with fatty acid chains of phospholipids, which decreases the fluidity of the plasma membrane, therefore it can modify the neuronal structure and synaptic (Zhang and Liu 2015). Other possibility is the cholesterol supplementation has favored the synaptic transmission, since this transmission is cholesterol dependent, what would explain the increase in brain activity (Martín et al. 2014). Also it is reasonable to think that the increase in the amount of cholesterol in the myelin sheath, region with the highest cholesterol concentration in the brain, has enhanced electrical insulation capacity of myelin sheath. This increase in the lipid content of the myelin sheath could increase the velocity of action potentials, thus increasing the brain electrical activity (Moyes and Schulte 2007).

Chali et al. (2015) also observed that an increase in brain cholesterol levels caused increase in brain activity, inhibition and death of interneurons and also increased the tau protein expression. Nevertheless, we noted that hypercholesterolemic diet was able to increase brain electrical activity without apparent neuronal damages.

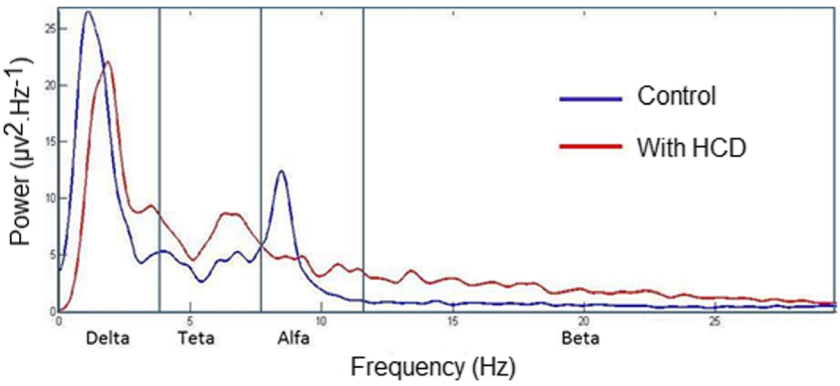

Fig. 3 Power spectrum of control animals and animals fed with hypercholesterolemic diet

Table 1 Average power values (μv2.Hz-1) of the different brain waves of animals without and with hypercholesterolemic diet (HCD), before (Basal) and submitted to *status epilepticus* (SE)

Group	Delta		Theta		Alpha		Beta	
	Basal	SE	Basal	SE	Basal	SE	Basal	SE
Without HCD	13.74 ± 2.37[a]	11.96 ± 5.02	6.40 ± 1.80	6.78 ± 1.90	2.77 ± 1.03	2.53 ± 1.01[a]	0.91 ± 0.21 [a]	1.23 ± 0.37 [a]
With HCD	10.12 ± 2.21 [b]	8.01 ± 3.74	6.78 ± 0.85	8.09 ± 2.51	3.20 ± 0.89	3.48 ± 0.91[b]	1.10 ± 0.16[b]	1.67 ± 0.57 [b]

Different letters in the same column for the same wave represent significant statistical difference. The values are represented as median and interquartile range.

However, studies are needed in order to understand the neuronal effects of the diet on people health. The hypercholesterolemic diet effect on brain waves was similar to that found in animals in *status epilepticus*. The hypercholesterolemic diet caused increased cerebral excitability, this excitability was more significant in animals fed with hypercholesterolemic diet and simultaneously submitted to *status epilepticus*. So we can regard that cholesterol maybe is involved in trigger of epileptic seizures. During epilepsy, seizures are only established when the neuronal excitation exceeds a critical threshold. We can regard that individuals who were fed with diets high in cholesterol and saturated fats had a higher cerebral excitability. This condition may promote the overcoming of this critical threshold in epileptic patients and thus favor the manifestation of epileptic seizures. In this way, hypercholesterolemic diets may represent a risk factor for epileptic individuals.

Some neurological diseases are being associated with problems in cholesterol metabolism, such as Alzheimer's disease and Niemann-Pick type C disease, showing changes in the brain electrical activity (Sevin et al. 2007; Panchal et al. 2010; Di Paolo and Kim 2011). The development of studies is important to evaluate the impact of these diets high in cholesterol and saturated fats on health, once the great increase in the consumption of these diets can affect the brain electrical activity.

Conclusion

Hypercholesterolemic diet caused increased brain excitability without neuronal damage. However, the animals fed with hypercholesterolemic diet and submitted to *status epilepticus* presented a higher brain excitability compared to control and animals in *status epilepticus* without diet. In conclusion, the hypercholesterolemic diet favored a greater severity of the *status epilepticus*.

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Compliance with ethical standards

Conflict of interest The authors state there are no conflicts of interest.

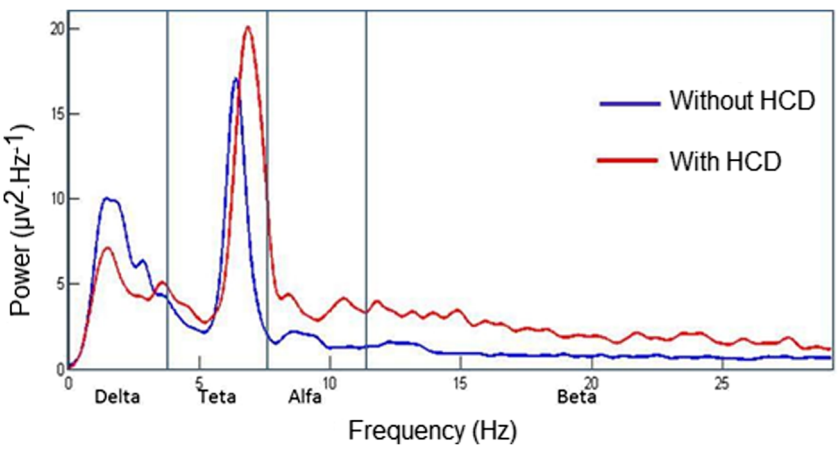

Fig. 4 Power spectrum of animals without and with hypercholesterolemic diet at *status epilepticus*

Statement on the welfare of animals All procedures performed in this study involving animals were in accordance with the ethical standards of the UFRPE, approved by the Local Committee for the Care and Ethical Use of Animals in Research (CEUA/UFRPE, Recife, PE, Brazil).

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